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L2: Entry 1 of 1

File: USPT

Aug 3, 1999

DOCUMENT-IDENTIFIER: US 5932210 A

TITLE: Recombinant adenoviral vector and methods of use

Drawing Description Paragraph Right (7):

FIGS. 7A and 7B show in vivo tumor suppression and increased survival time with A/M/N/53. H69 (SCLC) tumor cells were injected subcutaneously into nude mice and allowed to develop for 2 weeks. Peritumoral injections of either buffer alone (---), control A/M adenovirus (-x-x-), or A/M/N/53 (-.DELTA-.DELTA.), both viruses (2.times.10.sup.9 pfu/injection) were administered twice per week for a total of 8 doses. Tumor dimensions were measured twice per week and tumor volume was estimated as described in Experiment No. II. A) Tumor size is plotted for each virus versus time (days) post inoculation of H69 cells. Error bars indicate the mean tumor size +/-SEM for each group of 5 animals. Arrows indicate days virus injections. B) Mice were monitored for survival and the fraction of mice surviving per group versus time post inoculation of buffer alone (----), control A/M (... ..) or A/M/N/53 (----) virus treated H69 cells is plotted.

Detailed Description Paragraph Right (28):

As shown in detail below, the recombinant adenoviruses expressing a tumor suppressor wild-type p53, as described above, can efficiently inhibit DNA synthesis and suppress the growth of a broad range of human tumor cell types, including clinical targets. Furthermore, recombinant adenoviruses can express tumor suppression genes such as p53 in an in vivo established tumor without relying on direct injection into the tumor or prior ex vivo treatment of the cancer cells. The p53 expressed is functional and effectively suppresses tumor growth in vivo and significantly increases survival time in a nude mouse model of human lung cancer.

Detailed Description Paragraph Right (35):

As with the use of the tumor suppressor genes described previously, the use of other anti-tumor genes, either alone or in combination with the appropriate therapeutic agent provides a treatment for the uncontrolled cell growth or proliferation characteristic of tumors and malignancies. Thus, this invention provides a therapy to stop the uncontrolled cellular growth in the patient thereby alleviating the symptoms of the disease or cachexia present in the patient. The effect of this treatment includes, but is not limited to, prolonged survival time of the patient, reduction in tumor mass or burden, apoptosis of tumor cells or the reduction of the number of circulating tumor cells. Means of quantifying the beneficial effects of this therapy are well known to those of skill in the art.

Detailed Description Paragraph Right (50):

Approximately 1.times.10.sup.7 H69 (SCLC) tumor cells in 200 .mu.l volumes were injected subcutaneously into female BALB/c athymic nude mice. Tumors were allowed to develop for 2 weeks, at which point animals were randomized by tumor size (N=5/group). Peritumoral injections of either A/M/N/53 or the control A/M adenovirus (2.times.10.sup.9 pfu/injection) or buffer alone (1% sucrose in PBS) were administered twice per week for a total of 8 doses/group. Tumor dimensions and body weights were measured twice per week for 7 weeks, and tumor volume was estimated as described previously. Animals were then followed to observe the effect of treatment on mouse survival.

Detailed Description Paragraph Right (57):

To address the feasibility of gene therapy of established tumors, a tumor-bearing nude mouse model was used. H69 cells were injected into the subcutaneous space on the right flank of mice, and tumors were allowed to grow for 2 weeks. Mice then received

peritumoral injections of buffer or recombinant virus twice weekly for a total of 8 doses. In the mice treated with buffer or control A/M virus, tumors continued to grow rapidly throughout the treatment, whereas those treated with the A/M/N/53 virus grew at a greatly reduced rate (FIG. 7A). After cessation of injections, the control treated tumors continued to grow while the p53 treated tumors showed little or no growth for at least one week in the absence of any additional supply of exogenous p53 (FIG. 7A). Although control animals treated with buffer alone had accelerated tumor growth as compared to either virus treated group, no significant difference in body weight was found between the three groups during the treatment period. Tumor ulceration in some animals limited the relevance of tumor size measurements after day 42. However, continued monitoring of the animals to determine survival time demonstrated a survival advantage for the p53-treated animals (FIG. 7B). The last of the control adenovirus treated animals died on day 83, while buffer alone treated controls had all expired by day 56. In contrast, all 5 animals treated with the A/M/N/53 continue to survive (day 130 after cell inoculation) (FIG. 7B). Together, this data establish a p53-specific effect on both tumor growth and survival time in animals with established p53-deficient tumors.

Detailed Description Paragraph Right (62):

Work presented here and by other groups (Chen et al. (1990); Takahashi et al. (1992)) have shown that human tumor cells lacking expression of wild-type p53 can be treated ex vivo with p53 and result in suppression of tumor growth when the treated cells are transferred into an animal model. Applicants present the first evidence of tumor suppressor gene therapy of an in vivo established tumor, resulting in both suppression of tumor growth and increased survival time. In Applicants' system, delivery to tumor cells did not rely on direct injection into the tumor mass. Rather, p53 recombinant adenovirus was injected into the peritumoral space, and p53 mRNA expression was detected within the tumor. p53 expressed by the recombinants was functional and strongly suppressed tumor growth as compared to that of control, non-p53 expressing adenovirus treated tumors. However, both p53 and control virus treated tumor groups showed tumor suppression as compared to buffer treated controls. It has been demonstrated that local expression of tumor necrosis factor (TNF), interferon- γ), interleukin (IL)-2, IL-4 or IL-7 can lead to T-cell independent transient tumor suppression in nude mice (Hoch et al. (1992)). Exposure of monocytes to adenovirus virions are also weak inducers of IFN- α /. β . (reviewed in Gooding and Wold (1990)). Therefore, it is not surprising that some tumor suppression in nude mice was observed even with the control adenovirus. This virus mediated tumor suppression was not observed in the ex vivo control virus treated Saos-2 tumor cells described earlier. The p53-specific in vivo tumor suppression was dramatically demonstrated by continued monitoring of the animals in FIG. 10. The survival time of the p53-treated mice was significantly increased, with 5 out of 5 animals still alive more than 130 days after cell inoculation compared to 0 out of 5 adenovirus control treated animals. The surviving animals still exhibit growing tumors which may reflect cells not initially infected with the p53 recombinant adenovirus. Higher or more frequent dosing schedules may address this. In addition, promoter shutoff (Palmer et al. (1991)) or additional mutations may have rendered these cells resistant to the p53 recombinant adenovirus treatment. For example, mutations in the recently described WAF1 gene, a gene induced by wild-type p53 which subsequently inhibits progression of the cell cycle into S phase, (El-Deiry et al. (1993); Hunter (1993)) could result in a p53-resistant tumor.

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L4: Entry 1 of 1

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Mar 6, 2001

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TITLE: Suppression of tumor growth by a mini-E1A gene

Brief Summary Paragraph Right (12):

The neu oncogene, which encodes a p185 tumor antigen, was first identified in transfection studies in which NIH 3T3 cells were transfected with DNA from chemically induced rat neuroglioblastomas (Shih et al., 1981). The p185 protein has an extracellular, transmembrane, and intracellular domain, and therefore has a structure consistent with that of a growth factor receptor (Schechter et al., 1984). The human neu gene was first isolated due to its homology with v-erbB and EGF-r probes (Senba et al., 1985). Molecular cloning of the transforming neu oncogene and its normal cellular counterpart, the neu proto-oncogene, indicated that activation of the neu oncogene was due to a single point mutation resulting from one amino acid change in the transmembrane domain of the neu encoded p185 protein (Bargmann et al., 1986; Hung et al., 1989). The neu oncogene is of particular importance to medical science because its presence has been correlated with the incidence of cancers of the human breast and female genital tract among others. Moreover, amplification/overexpression of this gene has been directly correlated with relapse and survival in human breast cancer (Slamon et al., 1987). Therefore, it is an extremely important goal of medical science to evolve information regarding the neu oncogene, particularly information that could be applied to reversing or suppressing the oncogenic progression that seems to be elicited by the presence or activation of this gene. Unfortunately, little has been previously known about the manner in which one may proceed to suppress the oncogenic phenotype associated with the presence of oncogenes such as the neu oncogene.

Detailed Description Paragraph Right (3):

The E1A gene generally produces two major spliced products, the 12S and 13S mRNAs, that encode proteins 243 and 289 amino acids long, respectively (Moran et al., 1987). To determine which E1A gene product was responsible for the observed repression, the same studies were performed with recombinant plasmids expressing either 12S or 13S E1A gene product (pE1A-12S and pE1A-13S). Hung et al. have previously shown that both the 12S and 13S products were effective at repressing neu transcription in a concentration-dependent manner. These E1A gene products contain at least two of three highly conserved regions referred to as CR1, CR2, and CR3 (Moran et al., 1987; Van Dam et al., 1989). In particular, while CR1 and CR2 exist in both the 12S and 13S, CR3 is unique to the 13S product. Since 12S itself can itself repress oncogenesis, Hung et al. reasoned that the CR3 is dispensable for suppression. However, the present inventors have discovered that other portions of E1A, which are found in both the 12S and 13S wild-type gene products, are nevertheless dispensable for tumor suppression and that various mini-E1A genes as described herein can effectively suppress tumorigenesis and enhance long-term survival in vivo.

Detailed Description Paragraph Right (163):

As noted above, the HER-2/neu oncogene encodes a MW 185,000 epidermal growth factor receptor-related transmembrane protein (p 185) with intrinsic tyrosine kinase activity. Overexpression of the normal human HER-2/neu proto-oncogene, which can also lead to higher overall tyrosine kinase activity, is a frequent event in many types of human cancers, including cancers of the breast, ovarian, lung, uterine cervix, stomach and colon cancer, for example. Correlation between the overexpression of HER-2/neu and the number of lymph node metastases in breast cancer patients and decreased survival in both breast and ovarian cancer patients has been reported. Hung et al. have shown that adenovirus 5 E1A gene product can repress HER-2/neu oncogene expression and suppress the tumorigenic and metastatic potential of activated rat neu oncogene-transformed mouse 3T3 cells. Introduction of the E1A gene into the human ovarian cancer cell line

SKOV-3(i.p.), which has enhanced expression of HER-2/neu, resulted in reduced malignant phenotypes in vitro and in vivo. Those data indicated that the E1A gene can be considered as a tumor suppressor gene for human cancer cells.

Detailed Description Paragraph Right (167):

SKOV-3(i.p.) (10.sup.6 /mouse) was transplanted into the peritoneal cavity of nu/nu mice. Five days later they received an intraperitoneal injection of viral solution (titer: 2.times.10.sup.9 PFU/ml) from either Ad.E1A(+), Ad.E1A(-), or Just PBS for 3 days, followed by once/week for 4.5 months. Clinical observation and survival rates showed that Ad.E1A(+) significantly prolonged the survival time of the mice and some mice were kept tumor free. Immunohistochemical analysis indicated that Ad.E1A protein was expressed in tumor tissue after gene delivery in vivo and expression of HER-2/neu P185 protein was greatly suppressed.

Detailed Description Paragraph Right (181):

Mice may be examined for tumor signs and symptoms, and killed when they appear moribund. Mice treated with the E1A or LT plus the anti-cancer drug and/or emodin like tyrosine kinase inhibitors will be expected to have a longer survival time.

Detailed Description Paragraph Right (184):

Long-term survival studies to assess the tumor suppressor activity of mini-E1AN80 have now confirmed the ability of mini-E1AN80 to suppress tumor formation in mice as described in Example 1 (see FIG. 7). Results from both of these studies thus indicate that mini-E1AN80 appears to be useful, as is E1A, for tumor suppression in vivo.

Detailed Description Paragraph Right (186):

The protocol for tumor engraftment and lipid-DNA complex formation for the long-term survival studies were performed as described above. Five mice were used in each group. In these studies, the ovarian carcinoma cell line SKOV-3.ip1 was used to induce tumors in the mice. SKOV-3.ip1 is derived from the SKOV-3 cell line and correlates with more rapid progression of peritoneal carcinomatosis and a higher degree of malignancy than SKOV-3 cells.

Detailed Description Paragraph Right (187):

After 5 days of tumor cell growth, injection of treatment compounds began with three injections the first week and subsequent injections were given every 7 days. Mini-E1AN80 was administered in two lipid:DNA formulations, namely, 13 nmole lipid:1 .mu.g DNA and 1 nmole lipid:1 .mu.g DNA. In each formulation, each injection contained 15 .mu.g of DNA. Data from this experiment is presented as survival curves in FIG. 7.

Detailed Description Paragraph Right (188):

As can be seen in FIG. 8, injections with mini-E1AN80 significantly prolonged mouse survival in both 13:1 and 1:1 formulations (mini-E1AN80-13 vs. PBS, $P < 0.025$). The day on which the last surviving animal from the control group died was also the last day of treatment injections, indicated by an asterisk (*) in FIG. 7. At this time point, 8/10 animals that had received mini-E1AN80 injections were still alive, as compared to 0/5 of those that had received PBS injections. Even though treatment injections were discontinued after approximately 110 days, 4 out of 10 animals that received mini-E1AN80 injections were still alive after one year, long after all of the control animals had died.

Detailed Description Paragraph Right (189):

In another long-term study, tumor suppressor activity of mini-E1AN80 was compared to that of full-length E1A in mice bearing SKOV-3 tumors. The full-length E1A constructs were mixed with cationic lipids at a 13:1 ratio and the experiment was performed as described above. The resultant survival curves, shown in FIG. 8, reflect the same mini-E1AN80 data discussed above (FIG. 7) along with the full-length E1A data. These results indicate that mini-E1AN80 appeared to be about as potent in tumor suppression as full-length E1A.

Detailed Description Paragraph Right (191):

Long term survival studies using an additional mini-E1A construct provided additional information supporting the utility of mini-E1A formulations in the treatment of cancers.

Detailed Description Paragraph Right (193):

Long-term survival studies were performed using the E1A-Cterm mini-gene in a tumor engraftment model and DNA-lipid formulations as described above. In this study, it appears that the subclone of SKOV-3.ip1 used may have been even more aggressive in

tumor formation than the SKOV-3.ip1 parent used to induce tumors in the nude mice since no control mice survived for more than about 35 days. The protocol was otherwise as in the long-term study above except that: (i) 10 mice were used in each group receiving E1A formulations and 9 mice were used in the control group; (ii) 5% dextrose was used for control injections; and (iii) a lipid:DNA ratio of 10:1 was used for the treatment formulations however, as above, each injection contained 15 .mu.g of DNA. Survival curves from this experiment are shown in FIG. 9.

Detailed Description Paragraph Right (194):

The results, as shown in FIG. 9, revealed that mini-E1ACterm provides substantial tumor suppression and long-term survival to an extent similar to that of full-length E1A. Indeed, at the time when the last surviving mice that received dextrose injections died, 100% of the animals receiving mini-E1ACterm and full-length E1A were still alive. In addition, it should be noted that tumor cells in this experiment were very aggressive in that all 9 of the control mice had died by about day 35. At the time of death, the mice exhibited large tumor masses, clearly observable upon visual inspection. After 21 weeks, the 4 surviving mice that received either mini-E1ACterm or full-length E1A exhibited no signs of such tumor masses.

Detailed Description Paragraph Center (9):

Use of a Mini-E1A Gene (E1AN80) to Suppress Tumorigenesis in a Long-Term Survival Study

Detailed Description Paragraph Center (11):

Use of a Second Mini-E1A Gene (E1A-Cterm) to Suppress Tumorigenesis in a Long-Term Survival Study

Other Reference Publication (127):

Slamon, et al., "Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene," Science, 235:177-181, 1987.